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Lipiodol fertility enhancement: two-year follow-up of a randomized trial suggests a transient benefit in endometriosis, but a sustained benefit in unexplained infertility

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BACKGROUND: A New Zealand randomized trial has shown that lipiodol treatment enhances fertility with high short-term effectiveness for women with endometriosis. METHODS: An open randomized trial in a single-centre secondary- and tertiary-level infertility service assessing lipiodol flushing versus no intervention. A total of 158 women with unexplained infertility (62 women with mild endometriosis and 96 women with pure unexplained infertility) were evaluated at 24 months after trial entry. The main outcome measure was clinical pregnancy, assessed using a Cox proportional hazards regression model. RESULTS: There was a significant benefit in overall pregnancy rate following lipiodol [hazard ratio 2.0, 95% confidence interval (CI) 1.3–3.2]. Among women with endometriosis, the benefit in pregnancy rate seen in the first 6 months following lipiodol (hazard ratio 5.4, 95% CI 2.1–14.2) was not present at 6–24 months (hazard ratio 0.6, 95% CI 0.2–2.1). There was a more consistent effect of lipiodol on fertility throughout the 24-month follow-up among women with unexplained infertility (hazard ratio 2.0, 95% CI 1.1–3.5). CONCLUSIONS: Lipiodol flushing is effective at enhancing fertility not only for women with endometriosis, but also for those with pure unexplained infertility.

Keywords: endometriosis; lipiodol; oil-soluble contrast media; randomized trial; unexplained infertility

Introduction

Growing evidence has suggested a therapeutic, fertility-enhancing effect of a hysterosalpingogram with oil-soluble contrast media (OSCM) (Johnson *et al.*, 2006). The results of our randomized trial showed a pronounced therapeutic effect of lipiodol flushing at 6 months follow-up among women with endometriosis (Johnson *et al.*, 2004) and we speculated this might result from an immuno-biological fertility-enhancing effect either on the intraperitoneal environment or on the endometrial environment to enhance implantation (Johnson, 2005; Johnson *et al.*, 2005). Others have suggested that OSCM might simply reduce the latency to pregnancy rather than to increase the overall number of couples who eventually become pregnant (Steiner *et al.*, 2003).

Lipiodol flushing has now become a routine fertility treatment option in New Zealand for couples with unexplained infertility, especially where the women has mild endometriosis (Brent *et al.*, 2006), but numerous factors other than strength of supporting data determine the uptake of a new approach to fertility treatment (Johnson *et al.*, 2006).

Of 11 randomized control trials (RCTs) reporting on the fertility effects of tubal flushing (Johnson *et al.* 2005), most have reported only short-term outcomes at 4 months (Ogata *et al.*, 1993), 6 months (Alper *et al.*, 1986; De Boer *et al.*, 1988; Nugent *et al.*, 2002; Johnson *et al.*, 2004), 8 months (Yang *et al.*, 1989), 9 months (Rasmussen *et al.*, 1991), or 12 months (Letterie *et al.*, 1990; Spring and Barkan, 2000), with only two RCTs previously reporting outcomes beyond one year: Steiner *et al.* (2003) at 18 months and Lindequist *et al.* (1994) at 20–39 months. It does not appear that account has been taken of the possibility that, as time progresses, some participants allocated to no treatment may in fact undergo a flushing procedure, and that the data may be influenced by women undergoing other fertility treatments.

The objective of this analysis, on a cumulative basis up to 24 months following entry into our randomized trial (Johnson *et al.*, 2004), was to ascertain whether a fertility-enhancing effect of lipiodol was present, given that women might have chosen to undergo treatment with lipiodol or other fertility treatments from 6 to 24 months after trial entry.

Materials and Methods

Study design

A detailed description of our randomized trial protocol, in line with CONSORT criteria, was presented with the 6-month follow-up results of the randomized trial (Johnson *et al.*, 2004). The trial was an open parallel RCT of lipiodol flushing versus no intervention in two pre-defined subpopulations of women with unexplained infertility, one group of 62 women with known endometriosis and another group of 96 women with unknown endometriosis (pure unexplained infertility).

Follow-up at 24 months

Data for the follow-up stage 6–24 months were collected from study participants by a telephone consultation with a research assistant (between January and June 2005), after completion of 24 months from randomization and study entry. Dates of further pregnancies and whether further treatments had been undertaken were recorded.

Statistical methods

As women were followed for 24 months from entry to the randomized trial with no control over their treatment after 6 months and some loss to follow-up, a Cox proportional hazards regression model was used to assess the effect of lipiodol treatment. This model included the lipiodol treatment as a time-dependent factor. The event of interest was pregnancy. As this study was a follow-up from a previously published randomized trial, a second Cox proportional hazards regression model was also performed, where time was partitioned into two: first, up to 6 months (the follow-up time of the Johnson *et al.*, 2004, trial); second, 6–24 months. This model allowed the treatments and the time intervals to be modelled separately or in selected combinations. An intention-to-treat (ITT) analysis, assuming all women remained in their original treatment group and were followed for 2 years, was used to calculate the relative risk for clinical pregnancy and for live birth plus ongoing pregnancy (ongoing pregnancy defined as a viable pregnancy of gestation 12 weeks or more) in the two original treatment allocation groups. The primary imputation for pregnancy data in the ITT analysis was that all women lost to follow-up did not become pregnant, however, sensitivity analyses for the imputed pregnancy data (where all those lost to follow-up were all assumed to be pregnant and where half those lost to follow-up were assumed to be pregnant) were also performed.

Results

Participant flow

The original trial protocol allowed for completion of 6 months of follow-up without other treatment interventions, but participants were able to have further treatment without restrictions between 6 and 24-month follow-up phase. Fig. 1a and b shows the flow of participants to the 24-month follow-up. Among 73 women randomized to lipiodol treatment, 43 were known not to be pregnant at the 6-month follow-up; of these, 1 woman underwent a further lipiodol procedure, 21 underwent other fertility treatments and 2 women were lost to follow-up. Of the 85 women originally randomized to no intervention, 70 were known not to be pregnant at the 6-month follow-up, 3 women underwent a lipiodol procedure, 22 underwent other fertility treatments and 8 women were lost to follow-up.

Actual treatment analyses

The survival curves showing time-to-pregnancy for women in the two condition categories by treatment are shown in Fig. 2. When modelling over the full 24 months, there was no indication that the effect of treatment differed between the conditions, endometriosis and unexplained infertility ($\chi^2 = 0.2$, df = 1, $P = 0.7$), and also there was no indication of a difference in the proportion of pregnancies between the two conditions [hazard ratio 1.1, 95% confidence interval (CI) 0.7–1.7, $P = 0.7$]. The model showed an effect of lipiodol treatment with a hazard ratio of 2.0 (95% CI 1.3–3.2). However, as there was an indication of a difference in response to treatment between the two conditions at the end of the RCT (Johnson *et al.*, 2004), an assessment of the hazard ratios before and after this time showed an interaction ($\chi^2 = 5.4$, df = 1, $P = 0.02$). Hence, the 24-month follow-up data were re-analysed with the two time periods (0–6 and 6–24 months) being assessed separately. This re-analysis showed a strong treatment effect for those with endometriosis during the first 6 months and no effect after that time ($\chi^2 = 7.6$, df = 1, $P = 0.006$ for the effects at the two times being the same), while for those with unexplained infertility the treatment effect appeared similar over time ($\chi^2 = 0.1$, df = 1, $P = 0.8$). The treatment effects at the two time periods were summarized by hazard ratios as follows: hazard ratio 5.4 (95% CI 2.1–14.2) for women with endometriosis at 0–6 months; hazard ratio 0.6 (95% CI 0.2–2.1) for those with endometriosis at 6–24 months; hazard ratio 1.8 (95% CI 0.8–3.9) for women with unexplained infertility at 0–6 months; hazard ratio 2.2 (95% CI 0.9–5.4) for those with unexplained infertility at 6–24 months. As the unexplained infertility group had very similar estimates for both time periods, a better estimate was for the full 24 months (hazard ratio 2.0, 95% CI 1.1–3.5).

Between the 6 and 24-month follow-ups, 44 women had at least one further fertility treatment, 56 were recorded as having no further treatment and information on further treatment was not available for 10 women. To incorporate this into the model, 10 women with no information were censored at 6 months in the survival analysis and assumed to have had no further treatment in the ITT analysis. Also, the date of further treatment was unknown, other than it was beyond the end of the RCT, and so it has been assumed that the additional treatment was received half way between the end of the trial and the end of follow-up for that woman. With these assumptions, the additional fertility treatment had a significant hazard ratio 19 (95% CI 8–45). Based on simulations where the timing of the additional treatments was varied, the estimate of the effect of additional treatment changed little, always having a significant association with the onset of pregnancy. The addition of this variable had no effect on the early endometriosis hazard ratio but moved the later endometriosis hazard ratio and the unexplained infertility hazard ratio towards unity. Using the assumptions above, the hazard ratio for endometriosis after 6 months was 0.8 (95% CI 0.2–2.5), whereas for unexplained infertility it was 1.6 (95% CI 0.9–2.9).

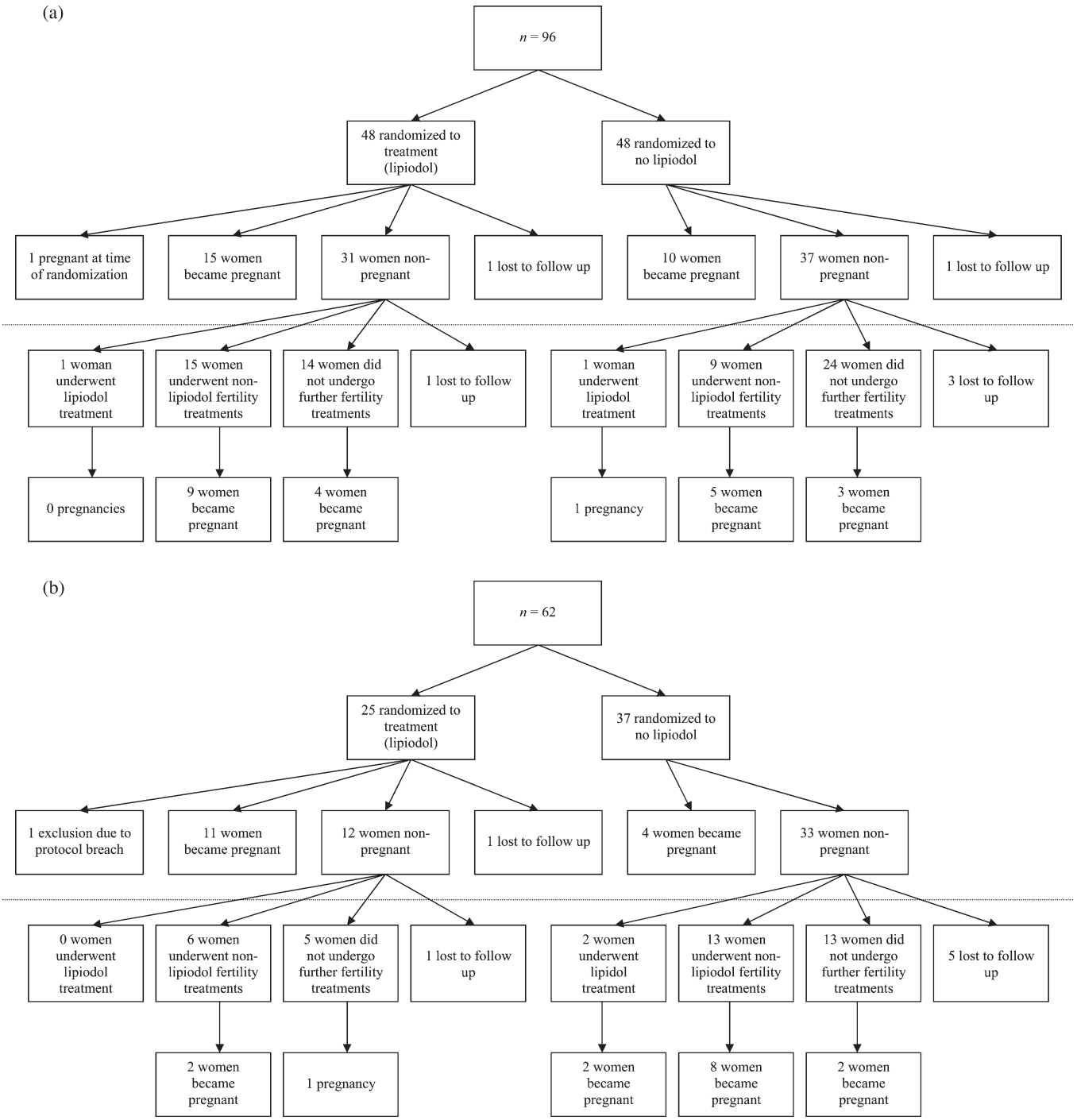

Figure 1: Flow of participants through the trial
Note in these figures that above the horizontal dotted line represents events from the FLUSH trial until the 6-month follow-up, and below the horizontal dotted line relate to events between 6 and 24 months of follow-up. (**a**) Flow of women with unexplained infertility to 24-month follow-up. (**b**) Flow of women with endometriosis to 24-month follow-up

Intention-to-treat analysis

Pregnancy with live birth and ongoing pregnancy rates at 24 months based on an ITT analysis according to group of allocation, and with an assumption that women lost to follow-up did not become pregnant, and sensitivity analyses for the imputed pregnancy data, are shown in Table 1. These analyses for pregnancy show very similar results to the Cox proportional hazards regression analysis but there are smaller relative risks because of the more conservative approach and the analyses for live births show very similar results to those for the pregnancy outcome.

Discussion

This 24-month follow-up of our randomized trial provides further evidence of the effectiveness of lipiodol flushing for

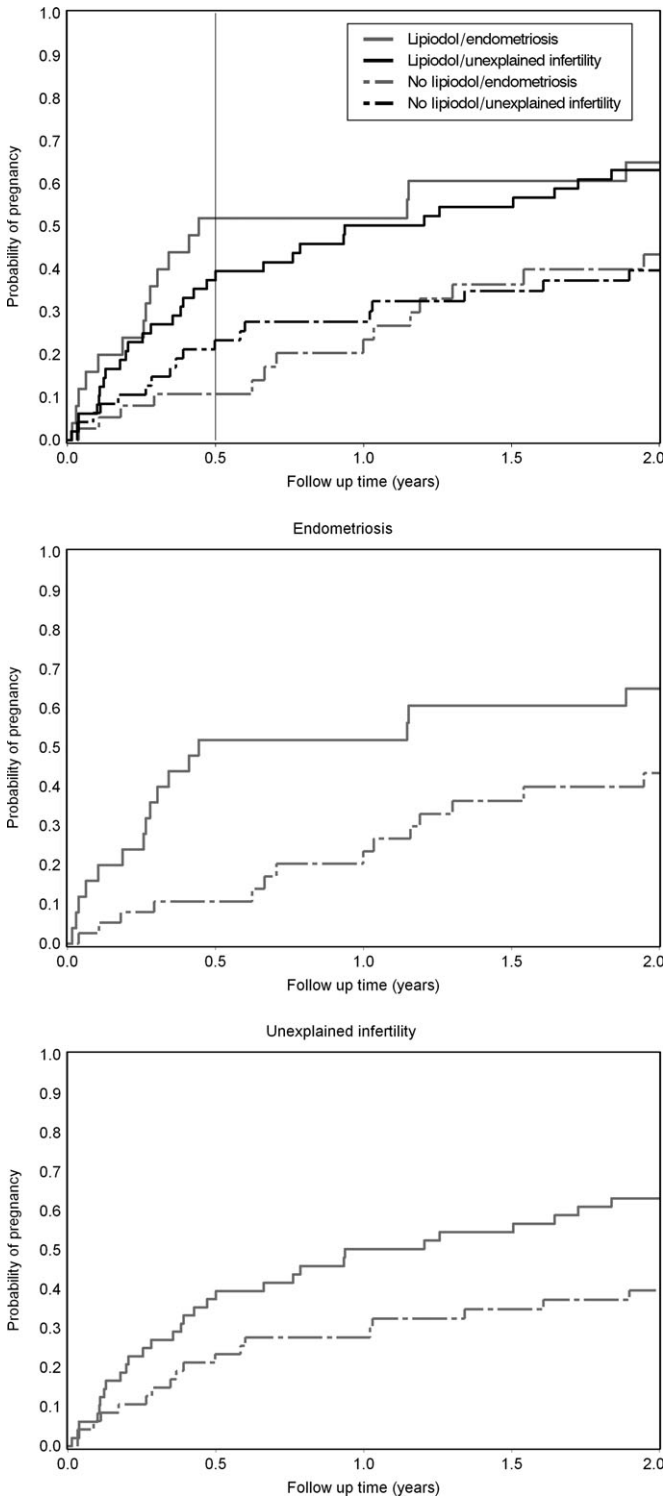

Figure 2: Survival curve of pregnancy over 24 months

women with unexplained infertility. While there was a positive effect of lipiodol in women with endometriosis at 6 months follow-up (Johnson *et al.*, 2004), this present analysis shows no evidence that enhanced fertility persists beyond 6 months in women with endometriosis, but suggests a sustained and consistent enhanced fertility up to 24 months in women with pure unexplained infertility.

There are obvious difficulties with the 24-month follow-up analysis. The data collection method for the 24-month follow-up was open to possible recall bias, but it was not possible to verify further treatments and further pregnancies from the medical notes, since there was such fragmentation of care between private and public health systems that any particular set of notes would have been even more prone to bias owing to missing information. Further treatment was collected as a dichotomous variable, whereas in retrospect the study could have been strengthened by ascertainment of the actual timing of further treatment. The ITT analysis at 24 months is complicated by three women allocated to no intervention who have now had lipiodol procedures (all of whom became pregnant) and considerably more women among those randomized to both lipiodol and no intervention who have subsequently received other fertility treatments. The similar results of actual treatment and ITT analyses at 24 months, and the fact that analysis taking account of these further treatments did not substantially alter estimates of treatment effect (hazard ratios 1.6 versus 2.0 for unexplained infertility and 0.8 versus 0.6 for endometriosis, see *Results*), point to the results as being robust.

It could be argued that the disappearance by 24 months of the significant fertility enhancement from lipiodol in women with endometriosis at 6 months supports the concept suggested by Steiner *et al.* (2003) that only latency to pregnancy and not the eventual pregnancy rate is altered by lipiodol treatment. However, the picture is undoubtedly complicated by the number of women becoming pregnant from other fertility treatments between 6 and 24 months.

The apparent difference in effect of lipiodol for women with endometriosis compared with women with pure unexplained infertility is consistent with the notion that infertility related to mild endometriosis is a distinct entity and not just another type of unexplained infertility. Indeed, women with such endometriosis-related infertility have been shown to have approximately half the fecundity of women with pure unexplained infertility of similar duration (Jansen, 1986; Toma *et al.*, 1992; Johnson *et al.*, 2004), perhaps related to additional mechanisms such as immuno-biological adverse effects on fertility (Johnson, 2005). It is speculative to suggest that more than one mechanism of the fertility-enhancing effect of lipiodol might explain these different effects in women with endometriosis compared with women with unexplained infertility. Known immuno-biological dysfunction in women with endometriosis, in conjunction with the pronounced early effect of lipiodol, which later disappears, might suggest an immuno-biological mechanism of lipiodol. An intraperitoneal effect that could influence oocyte quality or sperm–oocyte interaction is plausible, but we have increasing evidence of an endometrial effect of lipiodol, which, in a murine model, had an effect on uterine dendritic cell populations (Johnson *et al.*, 2005). It is possible that lipiodol has an implantation enhancing effect on the endometrium, a hypothesis that we are currently investigating further. Whether the more sustained effect of lipiodol in women with pure unexplained infertility might be due to another mechanism, such as mechanical flushing of the fallopian tubes, remains unclear.

Table 1: ITT analysis of follow-up data at 24 months

	Unexplained infertility				Endometriosis-related infertility				Total population			
	Lipiodol ($n = 48$)	No flush ($n = 48$)	Relative risk (95% CI)	*P*-value	Lipiodol ($n = 25$)	No flush ($n = 37$)	Relative risk (95% CI)	*P*-value	Lipiodol ($n = 73$)	No flush ($n = 85$)	Relative risk (95% CI)	*P*-value
Clinical pregnancy	29	19	1.5 (1.0–2.3)	0.04	14	16	1.3 (0.8–2.2)	0.32	43	35	1.4 (1.0–2.0)	0.03
Sensitivity analyses of imputation for clinical pregnancy												
(i)	31	23	1.4 (0.9–1.9)	0.11	17	21	1.2 (0.8–1.8)	0.36	48	44	1.3 (1.0–1.7)	0.08
(ii)	30	21	1.4 (1.0–2.1)	0.07	16	19	1.3 (0.8–1.9)	0.31	46	40	1.3 (1.0–1.8)	0.05
Live birth plus ongoing pregnancy	25	15	1.7 (1.0–2.8)	0.05	12	12	1.5 (0.8–2.8)	0.21	37	27	1.6 (1.1–2.4)	0.02
Ectopic pregnancy	1	1			0	0			1	1		
Multiple pregnancy	2[a]	0			0	1			2[a]	1		

Assumption for ITT analysis: those lost to follow-up did not become pregnant. Sensitivity analyses of imputation for clinical pregnancy: assumption (i) that all those lost to follow-up became pregnant; assumption (ii) that half of those lost to follow-up became pregnant. Clinical pregnancy was assessed at 24 months post-randomization; pregnancy outcomes were subsequently ascertained for women achieving pregnancy by that time. [a]These two multiple pregnancies were achieved through IVF from 6 to 24 months follow-up.

Conclusion

This study lends further support to the effectiveness of lipiodol flushing for treating unexplained infertility. While previous evidence suggested the greatest short-term benefit is apparent in women with endometriosis, this study provides compelling evidence of more sustained efficacy for women with pure unexplained infertility.

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